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Evaluation of Sterilization By Gaseous Ethylene Oxide

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Evaluation of Sterilization By Gaseous Ethylene Oxide

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Public Health Monograph No. 68

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Introduction

In medical practice and research, sterilization of materials and equipment is frequently necessary to prevent infection. Steam pressure and other heat sterilization methods are usually satisfactory; however, a number of materials, certain instruments, and various types of apparatus are easily damaged by the high temperatures needed for steam or heat sterilization. Recent developments in automatic equipment utilizing gaseous ethylene oxide at lower temperatures now make practical the sterilization of many heat labile objects used in hospitals and laboratories (*1–5*). This paper presents an evaluation of a new method of sterilization, which uses a nonflammable, nonexplosive mixture of ethylene oxide and halogenated hydrocarbons with low pressure characteristics in an automatically controlled sterilizer.

Ethylene oxide has been known for many years to have bactericidal properties. Cotton and Roark (*6*), while testing fumigants, observed the lethal effect of ethylene oxide on a number of insects. Phillips and Kaye (*7*) reported that the first reference to antibacterial action appeared in 1929, in a patent application by Schrader and Bossert. Pappas and Hall (*8*), Yesair and Williams (*9*), and Kirby, Atkin, and Frey (*10*) reported the successful use of ethylene oxide in the control of microbial contaminants in spices and baked goods.

EFFECT OF CONCENTRATION OF GAS. Velu, Lepigre, and Bellocq (*11*) sterilized glass, metal, and cloth surfaces by exposing them to ethylene oxide. Phillips (*12*) reported that doubling the concentration of ethylene oxide reduced the sterilization time about one-half, and that the bactericidal activity was increased by a factor of 2.74 for each 10° C. rise in temperature within the range of 5° to 37° C.

EFFECT OF RELATIVE HUMIDITY. Kaye (*13*) found ethylene oxide effective against spores of *Bacillus subtilis* var. *globigii* in aerosol studies. Kaye and Phillips (*14*) showed that humidity was an important factor in the killing action of ethylene oxide. Their studies indicated that *B. subtilis* var. *globigii* spores at 28 percent relative humidity were killed 4 times as rapidly as at 65 percent relative humidity, and almost 10 times as rapidly as at 97 percent.

Kaye (*15*) reported that a gaseous mixture of 10 percent ethylene oxide and 90 percent carbon dioxide sterilized artificially contaminated blankets, linens, and books. Wilson (*16*) stated that plaster bandages exposed to ethylene oxide overnight were sterilized. Mathews and Hofstad (*17*), Klarenbeek and van Tongeren (*18*), and Callis and associates (*19*) have reported inactivation of several viruses after exposures to certain concentrations of ethylene oxide.

Skeehan, King, and Kaye (*20*) sterilized ophthalmological instruments with a mixture of ethylene oxide and dichlorodifluoromethane. Kaye and Darker (*21*) reported the sterilization of surgical, dental, and medical instruments by ethylene oxide vapor.

Nonflammable formulations for sterilizing sensitive materials were reported by Haenni and associates (*22*).

Description of Equipment

The sterilizer used for this study was an open mounted developmental model shown in figure 1.[1] This equipment consisted of a heated vacuum pressure type chamber, a vacuum pump, a charging manifold, and automatic controls, all mounted on a single stand enclosed in a stainless steel cabinet.

Interior chamber dimensions of 16 inches by 16 inches by 30 inches provided a sterilizer capacity of approximately 4½ cubic feet. The chamber was of single-walled, all-welded steel construction with baked enamel interior, vacuum pressure type designed for 33 pounds per square inch working pressure. Six electric strip heaters, each of 500 watt rating, were equally distributed on the two sides and bottom of the chamber for heating the sterilizing gas. These heaters were controlled thermostatically to maintain chamber temperatures varying from room temperature to 140° F.

An electrically driven vacuum pump connected directly to the chamber exhausted it to approximately 26 inches of mercury vacuum prior to charging it with sterilizing gas. This vacuum pump was also used to exhaust the gas to an atmospheric vent at the end of the automatic sterilizing cycle.

The charging manifold contained devices for adding water to humidify the chamber and for puncturing and maintaining a tight seal with either one or two disposable cans of sterilant.[2] It also included a solenoid valve to allow the water and the ethylene oxide gas mixture to enter the chamber as soon as a preset vacuum was reached.

The sterilizing cycle was operated automatically by a system of pressure-operated mercury switches, and an electric timer, relays, and solenoid valves. The timer had a selection of exposures up to 24 hours. A pressure and vacuum-temperature recorder with 6-inch diameter, 24-hour chart indicated and recorded chamber pressures and temperatures for each cycle. The electrical supply was 120 volts, 60 cycle a.c., single phase for the controls, and 208 volts, 60 cycle a.c., three phase for the heaters.

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[1] This sterilizer was purchased from the American Sterilizer Company, Erie, Pa. Improved production models of this type of equipment are available from this company and other manufacturers.

[2] A nonflammable, nonexplosive mixture of ethylene oxide (11 percent) and halogenated hydrocarbons (89 percent) with low pressure characteristics.

Method of Evalution

A variety of materials and apparatus together with representative test organisms was exposed to a range of conditions to determine the effect of the gas on the micro-organisms and exposed materials. Chamber pressure and temperature, time of exposure of load to gas, mechanical performance and other pertinent information were recorded for each test run. Temperature of selected positions within the sterilizer chamber was measured with thermocouples connected to a multipoint recording potentiometer (fig. 1). Concentrations of ethylene oxide and relative humidity of the gas mixtures in the sterilizer were measured with a gas chromatograph and an electric hygrometer.

Determination of Gas Concentration and Humidity. To obtain a rapid and fairly accurate method of determining ethylene oxide gas concentrations from small samples drawn from the sterilizer chamber, a gas chromatographic technique was selected. A system was developed to allow simultaneous determination of the relative humidity of the gas sample. The apparatus used is shown in figure 2. Gas samples were fed through copper tubing from the back of the sterilizer chamber to the gas chromatograph on the left side of the table. Approximately 1 milliliter of gas was required for each determination. The relative concentration of ethylene oxide was recorded by the adjustable span potentiometer on the right.

The apparatus shown in figure 2 included an electric hygrometer cell (on hotplate) for sensing humidity and temperature of the gas sample and a meter (in center of table) for indicating relative humidity, with accuracy of ±1½ percent. The barometer used to determine atmospheric pressure was on the left side of the sterilizer, while the gauge for measuring the gas pressure in the chamber was located on the right side of the sterilizer. The gas cylinder on the floor contained helium used in all tests as the carrier gas for the chromatographic apparatus. All calibrations and calculations required during the study were based on the ideal gas law.[3]

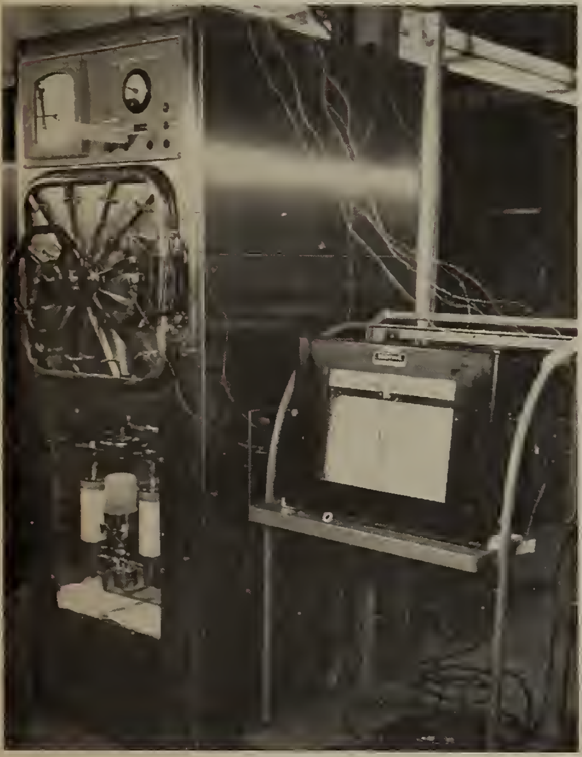

Figure 1. Automatically controlled gas sterilizer and multipoint recorder for temperature measurements.

Exposure of Micro-organisms. Several strains or species of micro-organisms were exposed to a mixture of 11 percent ethylene oxide and 89 percent halogenated hydrocarbons in this study. The predominant test organisms were the spores of *B. subtilis* var. *globigii*, since they are recognized as being much more resistant to lethal agents than nonsporeforming organisms. Other bacteria used were *Escherichia coli*, *Salmonella typhi*, *Mycobacterium phlei*, *Staphylococcus aureus*, 209; *Staphylococcus aureus*, phage type 7; a *Proteus* species, a *Pseudomonas* species, and *Bacillus stearothermophilus*. Filter paper strips impregnated with spores of *B. subtilis* var. *globigii*[4] were used extensively during this evaluation whenever material was not directly contaminated with the test organism. Controls in each lot of spore strips were tested to determine their viability. Pathogenic fungi[5] used in the study were *Candida albicans*, *Cryptococcus neoformans*, *Sporotrichum schenkii*, *Coccidioides immitis*, *Histoplasma capsulatum*, and *Blastomyces dermatitidis*.

In determining the microbicidal effect of the ethylene oxide-halogenated hydrocarbon mixture on the bacteria and pathogenic fungi, fungal cells in the pathogenic yeast phase and the saprophytic spore phase and bacterial cells were placed on sterile filter paper strips in sterile petri dishes and exposed to the ethylene oxide-halogenated hydrocarbon vapors in the chamber of the sterilizer. Inoculated control strips were also tested at room temperature and at the sterilizer chamber temperature to determine any lethal effects due to temperature or drying, or both. No controls were run to determine the effects of pressure and vacuum, since previous experience indicates that very

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[3] Details may be obtained from the Sanitary Engineering Branch, National Institutes of Health, Bethesda, Md.

[4] Supplied by American Sterilizer Company, Erie, Pa.

[5] Studies with pathogenic fungi were carried out in cooperation with Dr. C. W. Emmons, National Institute of Allergy and Infectious Diseases, Public Health Service.

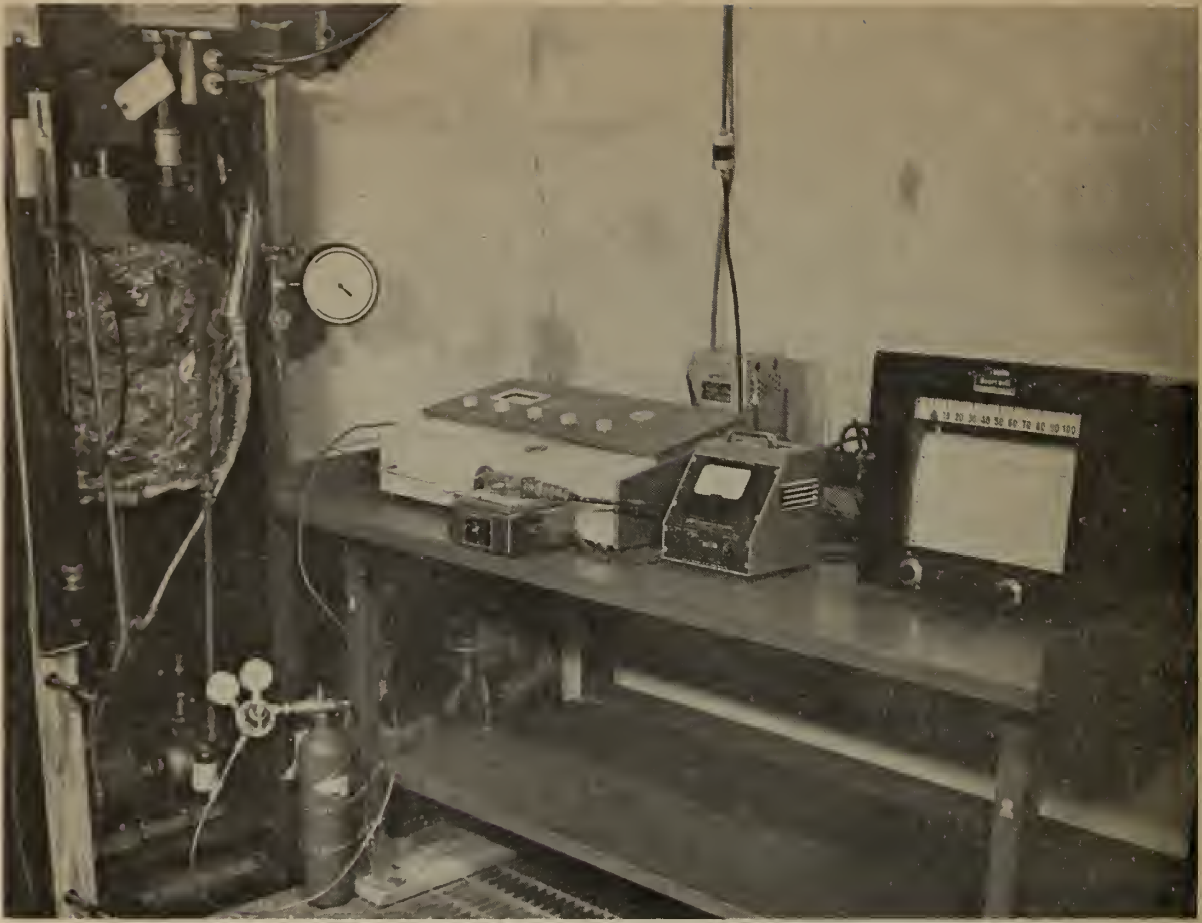

Figure 2. Apparatus for measurement of ethylene oxide concentration and relative humidity in sterilizer.

little, if any, lethal effect is exhibited by these two physical agents, especially in the ranges used for the apparatus tested.

When spore strips of *B. subtilis* var. *globigii* could not be used to obtain conclusive data, materials were contaminated with spore suspensions of this organism deposited in or on the following items: plastic tubing, hypodermic needles, urinary catheters, X-ray catheters, rubber surgical gloves, cystoscope, pharyngoscope, heart valve dilator, and heart valve knife. Spores for the inoculum were developed on nutrient agar until approximately 90 percent sporulation had occurred as determined by microscopic spore count. They were then harvested, washed, and suspended in buffered distilled water and heat shocked at 80° C. for 15 minutes to eliminate vegetative cells.

Concentrations of organisms in the inoculums used in these tests were determined by plate counts for vegetative and spore suspensions of bacteria and by microscopic spore counts for spores of the pathogenic fungi.

To determine survival of organisms after exposure to ethylene oxide gas mixture, the filter paper strips inoculated with bacterial spores or vegetative cells were transferred aseptically to tubes of fluid thioglycollate sterility testing medium. Items contaminated with *B. subtilis* var. *globigii* spore suspensions were rinsed with sterile buffered distilled water and the rinse placed in the thioglycollate medium. Preliminary tests indicated that organisms could be removed from items by the rinse technique. However, no tests were done to determine the percent recovery obtained. The tubes of thioglycollate containing the strips or the rinse were incubated at 37° C. for 7 to 10 days and observed for growth during incubation. In the case of the pathogenic fungi, the exposed filter paper strips containing the organisms were transferred to Sabouraud's maltose agar (Difco) and observed for growth during an incubation period of 14 days at 37° C.

Exposure of Materials. Materials being sterilized were subjected to an initial vacuum, exposure to ethylene oxide gas mixture under pressure for 2 or more hours at slightly elevated temperatures, and a final vacuum at the end of the exposure period. Because of these conditions and since ethylene oxide is a penetrating, reactive compound, it was problematical whether the exposed materials would be damaged during the sterilization cycle. To help answer this question, several types of materials were observed closely after each exposure for indications of any alteration in their physical properties.

Results

General Performance. The overall performance of the developmental gas sterilizer was observed for 18 months, during which it was subjected to 115 test runs and approximately 800 hours of operation. Several minor mechanical and electrical corrections were required during this period. In general, the process of sterilization was reliable, safe, and satisfactory.

Figure 3 shows a pressure-temperature chart for a typical 2-hour exposure of materials using two cans of sterilant. The pressure record indicates that 26 inches of mercury vacuum were reached prior to automatic injection of the liquid sterilant. As the sterilant changed from liquid to gas, the chamber pressure rose to 15 pounds per square inch gauge. At the end of the 2-hour sterilizing period, the gas was automatically evacuated to 24 inches of mercury vacuum. The indicated temperature range for this cycle was 132° F. and 140° F.

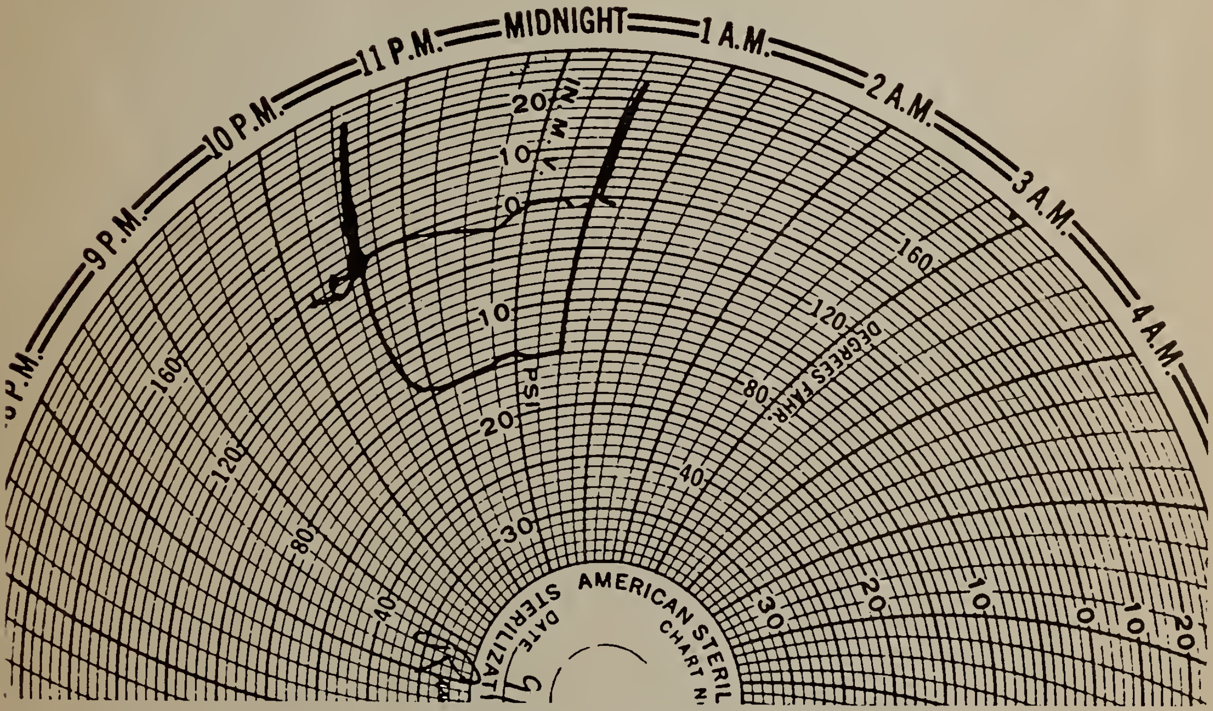

Figure 3. Pressure-temperature chart for typical 2-hour exposure of materials, using two cans of sterilant.

Pressure-temperature charts for typical 4-hour exposure of materials using one can of sterilant were similar to the chart shown in figure 3, except for chamber pressure which was 2 psig instead of 15 psig.

Chamber temperatures in the prototype sterilizer used for the tests varied considerably, as indicated by thermocouples placed throughout the equipment (*23*). Improvements were later made on the production models to obtain more uniform temperatures. In general, however, temperature control was adequate for sterilization of heat-sensitive materials without damage.

Concentration of Ethylene Oxide Gas in Chamber. Six sterilization tests were made, during which the concentration of ethylene oxide and the relative humidity were determined. Of the six, three were made using one can of sterilant each as a charge, the remaining three using two cans of sterilant each. During each period of sterilization, which lasted from 2 to 4 hours, four to eight samplings were taken. The results from each sampling were averaged over the entire run.

Figure 4 shows a typical chromatograph of a sample of gas taken from the sterilizer. The height of the peak corresponding to ethylene oxide was measured and multiplied by the attenuation factor to determine (from a calibration curve) the number of moles of gas present. Since the gas was at atmospheric pressure and the temperature different from that in the chamber, calculations had to be performed to give the concentration of gas under the chamber conditions. This was accomplished by assuming that the ideal gas law was obeyed and by using the measured values of temperature and pressure in the chamber and that of the gas just before it entered the chromatographic apparatus. These data together with the known volume of the sample loops (1.29 ml.) gave the weight of gas per unit volume in the chamber of the sterilizer itself.

Figure 4. Gas chromatogram of ethylene oxide, freons, and air in sample taken from sterilizer.

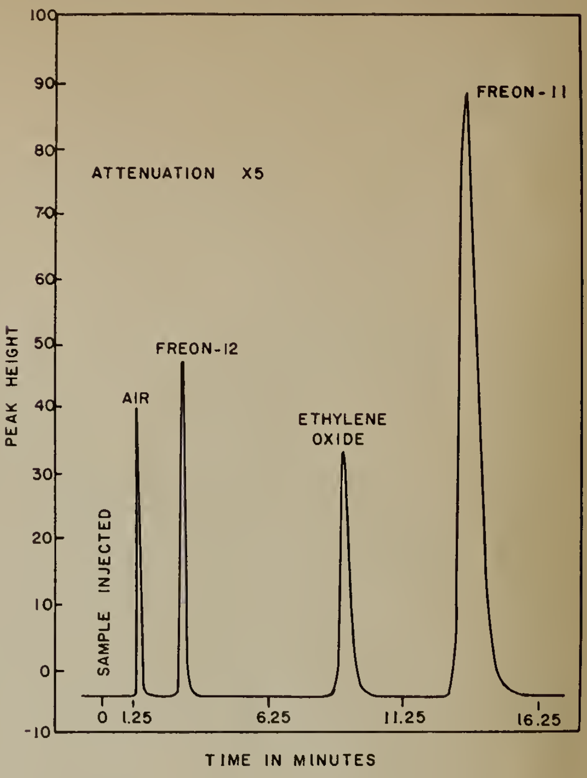

Table 1 gives the results obtained using one and two cans of sterilant. One may compare the experimental results with the values expected from the amount of sterilant charged into the chamber. The net weight of the gas was obtained by weighing the cans before and after the run. By estimating the volume of the chamber as 4.5 cubic feet and the percent by weight of ethylene oxide in the sterilant as 11 percent, the expected concentration of the ethylene oxide was calculated. The results of this calculation are given in table 1. Agreement between this calculation and experiment is very good. However, one should remember that the figure estimated for the volume is rough, since it does not take into account the curvature of the chamber and such volumes as the empty cans; hence, the excellent agreement may be fortuitous. One can have little doubt on the basis of these results that the contents of each can are volatilized during the cycle.

Relative Humidity of Gas Mixture in Chamber. Measurements of relative humidity were made of the gas samples just prior to their entry in the chromatographic apparatus. The values obtained were converted to weight of water in a given volume of saturated air at the temperature of measurement. By assuming that the water vapor followed ideal gas behavior, the relative humidity of the gas in the chamber was calculated.

Table 2 gives the average relative humidity for three runs using one can of gas each and for three experiments using two cans of gas each. Since the temperature of the chamber varies, the relative humidity will also vary. Therefore, the average value of the relative humidity was calculated by determining the weight of water in a given volume at the temperature and pressure of the chamber. This value was averaged for all the samples in a given run. In addition, the temperature of the chamber was averaged for the same samples. The weight of water in saturated air at the average temperature was obtained from tables (*24*), and by dividing this figure into the average value of the measured weight of water, the average relative humidity was calculated.

Table 2. Average relative humidity, using one and two cans of sterilant

Experiment No.	Average weight of water g/m³	Average temperature °F.	Average relative humidity (percent)
	One can of sterilant		
5	18. 5	124	21
7	18. 2	126	20
11	35. 5	126	39
	Two cans of sterilant		
8	28. 2	126	31
9	32. 9	126	36
10	44. 7	124	51

The values of the relative humidity in the chamber during experiments 5, 7, 8, and 9 were obtained from measurements of the gas at room temperature and atmospheric pressure. Under these conditions, the relative humidity which was measured was at the extreme upper limit of the sensing element. It was felt that these results should be viewed with a certain amount of doubt because of the decrease of sensitivity in this range. By raising the temperatures of the gas while making the measurement and not changing the water content of the gas, the relative humidity could be decreased into the range of the maximum sensitivity of the sensing element. This was accomplished by placing the steel chamber which held the hygrometer element on a thermostatically controlled hotplate and covering with insulation. In this way, the temperature of measurement was raised from 82°F. to approximately 120°F. and the measured relative humidity lowered from around 65 percent to 40 percent, which is in the range of maximum sensitivity. This modification was employed in experiments 10 and 11. The most weight should be given to the results of these

Table 1. Ethylene oxide concentration, using one and two cans of sterilant

Experiment No.	Number of samples	Average concentration ethylene oxide mg./l.	Average deviation mg./l.	Calculated concentration mg./l.
		One can of sterilant		
5	4	497	±5	517
7	4	487	±10	519
11	8	544	±23	522
Average for three runs		509	±13	519
		Two cans of sterilant		
8	5	887	±90	1, 048
9	5	1, 005	±155	1, 040
10	5	1, 099	±58	1, 048
Average for three runs		997	±101	1, 045

latter two experiments. These results indicate that the relative humidity in the chamber was in range of 30 percent to 50 percent.

EFFECT OF ETHYLENE OXIDE GAS ON MICRO-ORGANISMS. Experimental data obtained from the ethylene oxide gas sterilization method studied are shown in tables 3–6.

Table 3 shows the results of two different exposures of various representative bacteria on filter paper strips to the gaseous sterilant. In all cases, sterility was obtained with strips exposed to the ethylene oxide gas mixture. It was observed that the exposure of *S. typhi* and *Proteus* sp. to 135°F. for 4 hours resulted in sterility of some of the strips inoculated with these organisms. The bacteria used in this test represent organisms having varying resistances, but all are susceptible to the lethal activity of ethylene oxide. The inoculums used in these exposures were many more times that which would usually be found on general hospital equipment prepared for sterilization. These concentrations of organisms would more nearly represent grossly contaminated materials.

Table 4 summarizes the results of exposures of pathogenic fungi on filter paper strips to ethylene oxide. The fungi were represented by paper test strips containing the parasitic and saprophytic forms of these fungi, including infected tissues for three of the species. Exposures of these samples for 2 hours to the contents of two cans of gas were sufficient to kill all the fungi tested except *Sporotrichum*. To test whether the saprophytic form, or spore stage, of this organism was actually more resistant, spores were exposed to the gas on two more occasions. As the table shows, no viable spores were recovered from three additional exposures, thus indicating that this fungus is not truly resistant to the concentrations of ethylene oxide used in the sterilizer. It should also be noted that the species of *Histoplasma* and *Blastomyces* in the yeast phase and in infected tissue were killed by the 2-hour exposure to 135°F.

Both tables 3 and 4 definitely illustrate the microbicidal activity of the ethylene oxide-halogenated hydrocarbon mixture. These tests can be considered similar to exposing certain other materials contaminated with these micro-

Table 3. Results of exposure of bacteria on filter paper strips to ethylene oxide gas mixture

Micro-organism	Cells per strip	Results sterile strips, exposed strips		
		Ethylene oxide exposure	135° F. control	Room temperature control
Two cans of sterilant with 2-hour exposure [1]				
Escherichia coli	24 x 10^7	2/2	0/2	0/2
Salmonella typhi	20 x 10^6	2/2	0/2	0/2
Staphylococcus aureus, 209	4 x 10^7	2/2	0/2	0/2
Mycobacterium phlei	TNTC [2] on 1–100 dilution	2/2	0/2	0/2
Proteus sp	6 x 10^7	2/2	0/2	0/2
Pseudomonas	TNTC on 1–100 dilution	2/2	0/2	0/2
One can of sterilant with 4-hour exposure [3]				
Bacillus subtilis var. *globigii* (spores)	1.5 x 10^6	4/4	0/4	0/4
Mycobacterium phlei	1.0 x 10^6	4/4	0/4	0/4
Staphylococcus aureus, 209	6.0 x 10^6	4/4	0/4	0/4
Salmonella typhi	2.5 x 10^6	4/4	2/4	0/4
Proteus sp	2.2 x 10^6	4/4	3/4	0/4

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[1] Approximate test conditions: 26-inch mercury prevacuum; 15 psig chamber pressure; 120° to 140° F. chamber temperature; 1,000 mg./l. ethylene oxide gas concentration; 30 percent to 50 percent relative humidity

[2] TNTC—Too numerous to count.

[3] Approximate test conditions: Same as above except as follows: 2 psig chamber pressure; 500 mg./l. ethylene oxide gas concentration.

Table 4. Results of 2-hour exposure of pathogenic fungi on filter paper strips to ethylene oxide gas mixture, using two cans of sterilant

Type suspension and fungus	Cells per strip	Results		
		Ethylene oxide exposure	135° F. control	Room temperature control
Infected tissue				
Histoplasma	(1)	No growth	No growth	Growth.
Blastomyces	(1)	No growth	No growth	Growth.
Coccidioides	(1)	No growth	Growth	Growth.
Yeast phase				
Histoplasma	1.0×10^7	No growth	No growth	Growth.
Blastomyces	1.5×10^4	No growth	No growth	Growth.
Candida albicans	1.3×10^6	No growth	Growth	Growth.
Cryptococcus	1.8×10^6	No growth	Growth	Growth.
Saprophytic phase				
Histoplasma	1.0×10^4	No growth	Growth	Growth.
Blastomyces	4.0×10^4	No growth	Growth	Growth.
Coccidioides	7.0×10^4	No growth	Growth	Growth.
Sporotrichum	1.6×10^6	Growth	Growth	Growth.
	1.4×10^6	No growth		Growth.
	1.0×10^5	No growth		Growth.
	2.0×10^5	No growth		Growth.

(1) Not counted.

NOTE: See table 3 for approximate test conditions.

organisms to the vapors of ethylene oxide for sterilization.

Table 5 shows the results obtained on a variety of materials exposed to gas sterilization using *B. subtilis* var. *globigii* spore strips as sterility indicators. The penetrability of ethylene oxide gas was demonstrated by placing the spore strips in a cotton roll and at varying locations from the center outward. An exposure period of 2 hours to the contents of two cans of gas was sufficient to kill the spores on the strips even when the roll of cotton was sealed in a polyethylene plastic bag 2 mils thick. In the case of the wrapping materials, the spore strips were placed inside the various types of envelopes or wrapping materials. In no instance were viable spores recovered after exposure of the material to ethylene oxide. Good penetration was obtained even through the polyethylene envelope 2 mils thick.

In several ethylene oxide exposures of Tygon tubing used on a heart-lung blood oxygenating apparatus, a control tube containing spore strips of *B. subtilis* var. *globigii* was also exposed. The control tubing was 12 feet long, and the spore strips contained in glassine envelopes were located at intervals of 2 to 3 feet within the tubing. As seen in table 5, no viable spores were recovered from any of the strips removed from the tubing after exposure to the ethylene oxide gas mixture.

A problem confronting the libraries of many hospitals is the sterilization or disinfection of books used by patients having infectious diseases. To determine whether or not the ethylene oxide gas mixture was effective in sterilizing books, spore strips were placed between the pages of books which were then exposed to the gas mixture in the sterilizer chamber. Several spore strips were placed between pages near the front, near the back, and in the center of the books. During the exposures the books were either lying flat, placed atop one another, or standing on edge. The number of books per exposure varied from one to eight. The exposure period varied from 2 to 18 hours with two cans of gas, and was 4 hours with one can of gas.

The results in table 5 indicate that in books standing on an edge, spores are killed whether the exposure is for 2 hours with two cans of gas or 4 hours with one can of gas. Books standing on edge apparently afford an easier pathway for the gas to reach the surfaces of the pages. On the other hand, books lying flat required 4 hours' exposure with two cans of gas to kill the spores.

In addition to the above evaluation tests, this sterilizing procedure was used to sterilize X-ray catheters, a "radium gun," and personal effects of patients. The X-ray catheters and "radium gun" were in muslin-wrapped trays containing several spore strips of *B. subtilis* var. *globigii* beneath the wrapping.

The personal effects which were processed consisted of various games, plastic toys, hobby materials, books, phonograph records, and clothing items which could not be heat treated. The various items were contained in closed plastic bags when received for processing. When these bags were placed in the sterilizer chamber, they were opened slightly to allow rapid passage of gases during the vacuum and pressure cycles of the sterilizing process. At the time the bag was opened prior to exposure, several spore strips were distributed at random among the items. No bacterial growth was obtained from the spore strips (table 5).

Lensed instruments have long been a sterilization problem, especially those which contain electrical apparatus. A pharyngoscope and cystoscope were used in this study as being representative of the lensed instruments. Many surgical instruments contain delicate blades or small moving parts which are difficult to sterilize and still maintain the efficiency of the instrument. A heart valve knife and a heart valve dilator were used as representatives of these kinds of surgical instruments. The items were contaminated with spore suspensions of *B. subtilis* var. *globigii* in areas considered least accessible to the ethylene oxide vapor. The bacteriological results of tests using the above instruments are shown in table 6. No viable organisms were recovered from instruments for exposure periods of 2 hours with two cans of gas or 4 hours with one can of gas.

Catheters of various sizes were contaminated with spore suspensions of *B. subtilis* var. *globigii* and exposed to the ethylene oxide gas mixture for periods ranging from 2 to 4 hours. The conditions of the exposures and the results of

Table 5. **Sterilization of various materials by ethylene oxide gas mixture, using Bacillus subtilis var. globigii (1 x 10^6 spores) spore strips as sterility indicators** [1]

Material exposed	Cans of sterilant [2]	Hours exposed	Number strips	Results
Cotton rolls (nonabsorbant):				
1-pound size	2	2	4	No growth.
1-pound size (sealed in plastic bag)	2	2	1	No growth.
Wrapping materials:				
Bleached kraft paper envelopes	2	2	6	No growth.
Buck steribag envelope	2	2	6	No growth.
Cellulose casing	2	2	6	No growth.
Polyethylene envelope	2	2	6	No growth.
Muslin wraps	2	2	1	No growth.
Surgical rubber gloves	1	4	4	No growth.
Tygon tubing (5/16 inch inside diameter, 12 feet long) [3]	2	4	4	No growth.
Books:				
1 standing on end	2	2	1	No growth.
1 large lying flat	2	2	1	Growth.
5 lying flat	2	18	5	No growth.
4 lying flat	2	4	4	No growth.
3 lying flat	2	2	3	Growth in 1.
8 lying flat (atop one another)	2	3	8	Growth in 2.
8 lying flat (atop one another) [4]	2	4	8	No growth.
4 standing on end	1	4	4	No growth.
3 standing on end	1	4	3	No growth.
Heart or X-ray catheters in muslin-wrapped trays [3]	2	4	1	No growth.
Radium gun [3]	2	4	2	No growth.
Games, plastic toys, and phonograph records [4]	2	4	4	No growth.
Plastic toys, games, books, and various hobby materials	2	3	6	No growth.

[1] Controls for each test showed positive growth.
[2] See table 3 for approximate test conditions.
[3] Test run at least five times with same results.
[4] Test run three times with same results.

Table 6. Results of exposure of contaminated instruments, catheters, small bore tubing, and hypodermic needles to ethylene oxide gas mixture, using Bacillus subtilis var. globigii as indicator [1]

Materials exposed	Cans of sterilant [2]	Hours exposed	Inoculum spores per item	Results
Instruments (pharyngoscope, cystoscope, heart valve knife, and heart valve dilator).	2	2	4.5×10^{6}	No growth.
	1	4	1.8×10^{6}	No growth.
	1	4	1.5×10^{6}	No growth.
	2	3	1.5×10^{6}	No growth.
	2	2	1.5×10^{6}	No growth.
Catheters (X-ray Nos. 4, 6, 7, and 8; and urinary Nos. 14 and 22).	2	4	1.0×10^{6}	Growth on 3 of 6 items.
	2	2	4.5×10^{6}	Growth on 3 of 6 items.
	1	4	1.8×10^{6}	No growth.
	1	4	1.5×10^{6}	No growth.
	2	3	1.5×10^{6}	No growth.
	2	2	1.5×10^{6}	No growth.
Catheters (urinary Nos. 14 and 22)	2	2	4.5×10^{6}	No growth.
Plastic tubing, 0.5 and 1.0 mm. inside diameter	2	2	1.0×10^{6}	Growth on 0.5 mm. tube.
One end occluded by syringe and plunger [3]	2	3⅓	1.0×10^{6}	Growth on both tubes.
Plunger removed from syringe [4]	2	3	1.0×10^{6}	No growth.
Plastic tubing only, 0.5 mm. inside diameter [3]	2	2	4.5×10^{6}	No growth.
	1	4	1.8×10^{6}	No growth.
	1	4	1.5×10^{6}	No growth.
	2	3	1.5×10^{6}	No growth.
	2	2	1.5×10^{6}	No growth.
Hypodermic needles (22-, 20-, 19-, and 18-gauge in lengths ranging from 1½ to 3½ inches).	2	2	4.5×10^{6}	Growth in 7 of 13 items.
	2	3	1.5×10^{6}	Growth in 9 of 13 items.
	2	2	2.0×10^{6}	Growth in 1 of 19 items.
	1	4	1.8×10^{6}	Growth in 1 of 9 items.
	1	4	1.5×10^{6}	Growth in 2 of 12 items.

[1] Controls for each spore suspension showed positive growth.
[2] See table 3 for approximate test conditions.
[3] Test run twice with same results.
[4] Test run four times with same results.

the bacteriological sampling are shown in table 6. The concentration of spores in the inoculum was rather extreme, and it was observed that erratic results were obtained when the inoculum was approximately 4½ million spores per item. When the inoculum was decreased to less than 2 million spores per item, no viable spores were recovered, whether the items had been exposed to the contents of two cans of sterilant for 2 hours or one can for 4 hours.

The problem of sterilizing small diameter tubing was encountered when an attempt was made to sterilize 0.5-mm. and 1.0-mm. polyethylene tubing attached to 2-ml. hypodermic syringes. The tubing on each syringe was approximately 36 inches long. The interior of the tubes was contaminated with spore suspensions of *B. subtilis* var. *globigii* and exposed to the ethylene oxide gas mixture. The conditions of the exposures and the results of the bacteriological samplings are shown in table 6. In three tests the tubing was exposed with the plunger of the syringe in the syringe barrel. Under these conditions, viable spores were obtained from the 0.5-mm. tubing in all three exposures and once from the 1.0-mm. tubing. In four subsequent tests, the plunger of the syringe was removed from the barrel. In these instances, no viable spores were recovered from any of the tubing, but it is pointed out that the exposure time was increased from 2 to 3 hours. Further tests were conducted using 0.5-mm. tubing only with varying exposure times and concentrations of inoculum. No viable spores were recovered whether the exposure time was 2 hours with two cans of gas or 4 hours with one can of gas.

Sterility was observed even when the inoculum per tube was 4½ million spores. It is seen from this series of tests that nonsterility was found when one end of the tubing was occluded, but sterility was obtained when both ends of the tubing were unobstructed to allow free access of the gas to the interior surfaces of the tubes.

To determine further the ability of the ethylene oxide gas mixture to sterilize the interior of small tubes or small tubular openings, a series of tests was made using hypodermic needles of various diameters or gauges and differing lengths. The needles were 22-, 20-, 19-, and 18-gauge with lengths of 1½, 2, 3, and 3½ inches. The needles were contaminated with spore suspensions of *B. subtilis* var. *globigii* in concentrations from 1 million to 5 million per needle and exposed either to the contents of two cans of gas for 2 hours or one can of gas for 4 hours. Results of these tests are shown in table 6.

Effect of Gas on Exposed Materials. A variety of hospital and laboratory materials, such as instruments, plastic materials of several types, surgical rubber gloves, and books, were exposed to the ethylene oxide gas-halogenated hydrocarbon gas mixture for periods ranging from 4 to 200 hours without damage.

Plastic materials, such as a plastic heart valve, polyethylene tubing and bags, plastic catheters, toys, and phonograph records were examined for visible changes. The heart valve was exposed to ethylene oxide four times for a total of 10 hours. There was no apparent damage or warping of the plastic. The plastic tubing and catheters which were used in these studies showed no observable damage or alteration of their physical properties. Some of the plastic toys and records which were exposed for more than 4 hours showed some indications of warping, which was no doubt due to the prolonged exposure to the heated chamber and not to the effects of the ethylene oxide.

Rubber gloves sterilized by ethylene oxide in six sterilization cycles for a total of 17 hours' exposure appeared to be in nearly new condition.

The cystoscope and pharyngoscope used in the study were examined very closely after each exposure. The cystoscope was exposed six times for a total of 17 hours and the pharyngoscope seven times for a total of 19 hours. Examination revealed no apparent corrosion of the metal parts of the instruments, the lenses appeared clear, and there was no observable change in the lens cementing compounds used in these instruments.

Hazards and Problems

Assuming the sterilizer was installed in an unventilated room with a volume of 2,500 cubic feet, the concentration of ethylene oxide in the room air theoretically could reach 1,000 ppm if all the gas escaped into the room. According to Sax (*25*), this would be at least 10 times the maximum allowable concentration in a working atmosphere. Information by Lloyd and Thompson (*2*) indicates that a person can work in an atmosphere containing 250 ppm ethylene oxide in single exposures of 1 hour without being harmed. Fortunately, one of the first symptoms noticed on overexposure to ethylene oxide is irritation to the eyes and nose, which helps to warn of the presence of a hazardous concentration of the gas. It is obvious, however, that this type of sterilizer should be installed only in a well-ventilated room.

Of special interest was the amount of ethylene oxide left in the sterilizer after the terminal vacuum was drawn. This was obtained for two experiments with 119 and 130 mg./l. concentrations of residual ethylene oxide. Simple calculations show that one should expect around 25 mg./l. of ethylene oxide after final vacuum. The experiments show a difference by a factor of almost five. A possible explanation of this may be the fact that these experiments were performed while sterilized materials were in the chamber. The higher concentration may be a result of relatively slow diffusion of the gas from the objects and the bag in which they were placed. Since the vacuum operated for only a short time, removal of the gas may not have been complete. In an adequately ventilated room, the residual ethylene oxide gas would not be hazardous.

Surgical gloves were sterilized by the ethylene oxide gas mixture. With clean, dry gloves the ethylene oxide vapor contacts all surfaces of the gloves. In addition to this, ethylene oxide is absorbed by rubber, a circumstance which may facilitate the sterilizing procedure. Because of the absorption of the gas in rubber, it is imperative that rubber goods sterilized with ethylene oxide not be used for several hours after they have been removed from the sterilizer. Contact of the sterilized rubber goods with the skin prior to aeration may result in severe chemical burns. A waiting period after sterilization allows the absorbed gas to diffuse from the rubber. This precaution should also be followed when using leather goods sterilized with ethylene oxide.

With the exception of one run, sterilization of all contaminated hypodermic needles was not achieved. In seeking an explanation for these erratic results, consideration must be given to the large quantities of inoculums used in these tests. Although all the needles were contaminated in the same manner, there was no control over the dispersion of the suspension within the individual needles. Thus, in some needles a thick layer of the suspension may localize in an area and in this way the cells near the surface protect the cells deeper in the layer. This nonuniformity of dispersion is very likely the variable responsible for the inconsistent results. In actual practice, this protective influence would be found only in grossly contaminated material and not in properly cleaned needles. Well-cleaned needles would contain only a few hundred bacterial cells at the most, and it is apparent from the results of these tests that such needles would be sterilized by ethylene oxide, since needles containing more than 1 million spores were sterilized.

The sterility obtained for plastic tubing and the nonsterility indicated for needles of similar diameter may be explained as follows: In the plastic tubing, the inoculum is probably spread over a greater surface than in a needle. Also, ethylene oxide vapor will diffuse through the walls of the plastic tubing and can reach the layer of spore suspension from two sides. In the needle, only one side of the spore suspension is accessible to the gas. Therefore, ethylene oxide has a greater probability of reaching organisms inside the plastic tubing than those inside the needle.

Znamirowski, McDonald, and Roy (*26*) have recently published data indicating the difficulty of killing bacterial cells and spores with an ethylene oxide-halogenated hydrocarbon mixture when the organisms were dried on glass surfaces, whereas little difficulty was experienced with organisms dried on absorbent materials. They suggest that such results may be due to the relationship between the physical condition of the organism and the relative humidity of the exposure. It is logical that water vapor and the ethylene oxide gas would be more likely to penetrate to the surface of cells on porous materials than to cells on solid, nonabsorbent surfaces. The authors point out that their results "do not necessarily apply to whatever modifications may be introduced" on machines subsequent to their investigation. However, test results do indicate that sterilizing different materials may require modification of the so-called standard cycle, just as certain types of loads for steam sterilization require deviation from the standard steam cycle. It is suggested, therefore, that preliminary tests for sterilizing articles be conducted if the operator has not had experience sterilizing either a particular object or material or when recommendations for the correct sterilization cycle are not available.

Summary

Practical equipment with automatic cycle control and which uses gaseous ethylene oxide for sterilization of heat-sensitive apparatus and materials in hospitals and laboratories has been developed by private industry for commercial production. This paper covers the evaluation of the method of sterilization which employs a nonflammable, nonexplosive mixture of ethylene oxide (11 percent) and halogenated hydrocarbons (89 percent) with low-pressure characteristics.

The field testing of this sterilization method was conducted over a period of 18 months during which mechanical performance of the equipment, reliability, and safety were studied. Temperature of selected points within the sterilizer chamber were measured with thermocouples connected to a multipoint recorder. A rapid and fairly accurate method of determining ethylene oxide concentration of small samples of gas withdrawn from the chamber was developed using gas chromatographic apparatus. Relative humidity was simultaneously determined by use of an electric hygrometer. A variety of materials and apparatus together with representative test organisms was exposed to a range of conditions to determine the effect of the gas on micro-organisms and exposed materials.

The sterilization method used in this study was found to be safe and reliable when used in an adequately ventilated room.

The ethylene oxide-halogenated hydrocarbon mixture used in this study was found to be a satisfactory sterilizing agent when used with a pre-vacuum of approximately 26 inches of mercury and in a manner that insured adequate exposure of contaminated surfaces to the gaseous mixture. A minimum exposure period of 2 hours sterilized all surfaces easily accessible to the ethylene oxide gas in a concentration of approximately 1,000 milligrams per liter at chamber pressure of approximately 15 pounds per square inch gauge, temperatures 120° F. to 140° F., and relative humidity between 30 percent and 50 percent. A 4-hour exposure period was also found to be satisfactory for the above conditions with an ethylene oxide concentration of approximately 500 milligrams per liter and corresponding chamber pressure of approximately 2 pounds per square inch gauge.

Preliminary tests should be performed to obtain correct exposure conditions when attempting to sterilize items with surfaces not easily accessible to the ethylene oxide gas or when previous sterilization data for such materials are not available. All exposed materials, especially leather and rubber goods, should be allowed an 8- to 12-hour aeration period in a well-ventilated area before being used in contact with body tissues. Packaging or wrapping material should allow free penetration of ethylene oxide throughout the package and should also prevent poststerilization contamination.

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